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1,4-DIPHENYL SEMICARBAZIDE AS AN INSECTICIDE

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In the search for insecticidal materials among organic compounds, which has been the subject of toxicological studies at Sanford, Fla., 1,4-diphenyl semicarbazide showed promising results on many of the insects against which it was tested. A patent application on the compound as an insecticide has been filed by A. F. Freeman, of the Division of Insecticide Investigations. The application was based, in part, on certain of the results presented in this paper.

1,4-Diphenyl semicarbazide is a white or cream-colored crystalline solid, insoluble in water, and moderately soluble in alcohol and acetone. It can be ground to a fine powder for dusting or readily suspended in water for use as a spray. At present the compound is not manufactured at a price permitting its use as a commercial insecticide, but if sufficient demand arises to justify manufacturing the material in large quantities it may be possible to reduce the cost greatly. The samples of derris and lead arsenate used as standards of comparison were finely ground commercial products. The derris sample contained 4.5 percent of rotenone.

Insects Tested

Since many organic compounds are specific in their toxicity, it was considered that a few tests on a large number of species of insects would furnish more useful information than many replicated tests on a few species. This compound was tested on 14 species, representing the orders Lepidoptera, Coleoptera, Isoptera, and Orthoptera. The majority of the tests were made on leaf-feeding lepidopterous larvae, since the highly susceptible species were members of this order. The insects used and the foliage fed to each were as follows:

Insect

Foliage

American cockroach (Periplaneta americana (L.))
Bean leaf roller (Urbanus proteus (L.))
Cabbage looper (Autographa brassicae (Riley))

None
Bean
Collards

Insect (Continued)

Foliage

Colorado potato beetle (<u>Leptinotarsa decemlineata</u> (Say))	Eggplant
Cross-striped cabbage worm (<u>Evergestis rimosalis</u> (Guen.))	Collards
Diamondback moth (<u>Plutella maculipennis</u> (Curt.))	Do.
Greenhouse leaf tier (<u>Phlyctaenia rubigalis</u> (Guen.))	Do.
Hawaiian beet webworm (<u>Hymenia fascialis</u> (Cram.))	Swiss chard
Imported cabbage worm (<u>Pieris rapae</u> L.)	Collards
Melonworm (<u>Diaphania hyalinata</u> (L.))	Pumpkin
Rice weevil (<u>Sitophilus oryzae</u> (L.))	Wheat (seeds)
Southern armyworm (<u>Prodenia eridania</u> (Cram.))	Collards
Southern beet webworm (<u>Pachyzancla bipunctalis</u> (F.))	Swiss chard
Termites (<u>Reticulitermes</u> sp.)	None

In the preliminary Petri-dish tests beet leaves were used with the Hawaiian beet webworm and the southern beet webworm and squash foliage with the melonworm. However, the plants listed above were used in all other tests.

Details of the technique employed have been described in a previous paper by the authors (2) and are only briefly reviewed here.

Preliminary Petri-Dish Tests

Preliminary tests were made in Petri dishes to determine whether the material was toxic to insects and to select the species that were susceptible. Excised leaf sections dusted rather heavily with undiluted 1,4-diphenyl semicarbazide were fed to nearly full grown larvae of 10 species of leaf-feeding insects. For each test the dust was applied to two leaf sections by exposing the top and bottom surfaces to a falling column of dust in a settling chamber. At the same time an aluminum plate was exposed and the weight of the dust deposit on the leaf calculated by subtracting the original weight of the plate from its weight after exposure. As the purpose of these tests was to show merely the species of insects that were susceptible to the compound, no attempt was made to control the amount of chemical that was applied to the foliage, nor were comparisons made with commonly used insecticides. Each dusted leaf section was placed in a Petri dish with 12 to 15 larvae. Since two dishes were used per test the average number of insects per test was 25 to 30. The dishes were held at room temperature for 48 to 72 hours before the amount of feeding was estimated and mortality counts were made.

The data in table 1 show 1,4-diphenyl semicarbazide to be highly toxic to larvae of the greenhouse leaf tier, the Hawaiian beet webworm, the southern armyworm, and the southern beet webworm. Little mortality of the Colorado potato beetle and diamondback moth larvae was recorded, but the compound was very repellent to these insects and greatly restricted their feeding. Extending the duration of the test might have resulted in greater mortality. Although in the test against the melonworm there was a poor kill with moderate feeding, subsequent tests, to be discussed later, showed much greater mortality of this insect. In several tests the cabbage looper, the cross-striped cabbage worm, and the imported cabbage worm fed heavily with no apparent deleterious results.

Table 1.--Preliminary tests on the toxicity of foliage dusted
with 1,4-diphenyl semicarbazide in closed Petri dishes

Insect	Deposit	Estimated feeding on second day	Kill in 2 days
	Micrograms per sq. cm.		Percent
Cabbage looper	125	Much	0
	140	do.	0
Colorado potato beetle	140	Slight	3
Cross-striped cabbage worm	125	Much	0
	140	do.	0*
Diamondback moth	170	Slight	30
Greenhouse leaf tier	170	Slight	60
	170	do.	100
Hawaiian beet webworm	170	Slight	100*
Imported cabbage worm	155	Much	0
	230	do.	6
	340	do.	12
	355	do.	0
Melonworm	115	Moderate	16
Southern armyworm	140	Slight	83
	170	do.	83
Southern beet webworm	125	Much	76*
	170	Moderate	100

* Percent kill in 3 days.

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Fumigation Tests in Petri Dishes

To check the possibility that the mortalities recorded in table 1 had been caused by a fumigating action of the material, a second type of Petri-dish test was conducted with the greenhouse leaf tier, the Hawaiian beet webworm, the southern armyworm, and the southern beet webworm. Approximately 0.5 gram of chemical was confined in the top of several Petri dishes between two pieces of filter paper to prevent the insects from coming into direct contact with it. Larvae were placed in each dish and allowed to feed on untreated leaf sections at room temperature for 48 hours. All the insects fed heavily and suffered no apparent injury during a 2-day period; it was therefore assumed that the chemical had not killed by fumigation in the previous Petri-dish tests.

Comparison of Compound with Standard Insecticides in Petri-Dish Tests

Fourth instars of the southern armyworm and fifth instars of the southern beet webworm were used in a series of Petri-dish tests to compare the toxicity of equivalent deposits of 1,4-diphenyl semicarbazide and lead arsenate on excised foliage. Fifth instars of the Hawaiian beet webworm and the melonworm were used in another series in which derris instead of lead arsenate was used for comparison. Deposits of approximately 50, 100, 150, and 200 micrograms per square centimeter were used in these comparisons, but the exact amount of chemical applied could not be determined until the aluminum plate had been weighed.

The results in table 2 are based on a series of 3 tests using 25 to 30 insects per test. They show that each of the dusts gave considerable control of all insects, the control being more or less proportionate to the amount of chemical used. In no case was lead arsenate or derris markedly superior to 1,4-diphenyl semicarbazide, and against two insects the standard was less toxic. Against the Hawaiian beet webworm and the southern armyworm there was no great difference between 1,4-diphenyl semicarbazide and similar weights of the standard. However, the compound was somewhat more toxic to the melonworm than was derris, being more toxic at 52 micrograms than derris at 200 micrograms per square centimeter. The southern beet webworm was the most resistant of the four species, but again 1,4-diphenyl semicarbazide caused higher mortality in 3 days than did the standard insecticide.

Comparison of Compound with Standard Insecticides as Sprays on Potted Plants

Since 1,4-diphenyl semicarbazide was as toxic as derris or lead arsenate in the Petri-dish tests, and had no fumigating action, it was next employed as a spray on potted plants against fifth instars of the Hawaiian beet webworm, the melonworm, the southern armyworm, the southern beet webworm, and the greenhouse leaf tier.

Table 2.—Toxicity of 1,4-diphenyl semicarbazide as compared with a standard insecticide when dusted on foliage and fed to nearly full grown larvae of several species confined in Petri dishes

Insect	1,4-Diphenyl semicarbazide			Standard insecticide		
	Deposit	Feeding on third day	Kill in 3 days	Deposit	Feeding on third day	Kill in 3 days
	Micrograms per sq. cm.	Percent		Micrograms per sq. cm.	Percent	
Derris (4.5 percent rotenone)						
Hawaiian beet webworm	52 100 148 197	Slight do. do. do.	78 87 93 95	55 100 150 197	Slight do. do. do.	85 93 95 90
Melonworm	52 100 150 197	Slight do. do. do.	100 100 99 100	55 100 152 200	Slight do. do. do.	86 92 93 92
Lead arsenate						
Southern armyworm	52 100 147 195	Slight do. do. do.	87 99 97 100	52 101 155 195	Slight do. do. do.	90 97 99 99
Southern beet webworm	53 100 148 197	Moderate do. do. do.	71 78 89 94	52 101 155 197	Moderate Slight do. do.	55 64 71 78

One difficulty encountered in the testing of organic chemicals as insecticides has been the preparation of good spray suspensions. Most of the chemicals that have been used are not readily dispersed when mixed with water and either sink to the bottom of the container or float on the surface of the water. With 1,4-diphenyl semicarbazide it was necessary to use a wetting or dispersing agent. Saponin was finally selected as a suitable agent and was used at a concentration of 0.12 percent. The saponin, 1,4-diphenyl semicarbazide, and enough water to form a thin paste were ground together in a mortar, and when the combination was thoroughly mixed it was gradually diluted to the proper concentration with water. A cream-colored suspension resulted, which sprayed satisfactorily. Similar sprays were made with derris and lead arsenate for comparison with the 1,4-diphenyl semicarbazide.

Treatments were applied to potted plants at concentrations of 1, 2, 4, and 8 pounds per 100 gallons of water, the application being made with a compressed-air gun of the type commonly used in paint-spray outfits. To prevent a heavy loss of chemical from run-off, spraying was discontinued as soon as the droplets on the leaves began to coalesce. Two plants subjected to the same spray were included in each test. After the sprays had dried, approximately 15 insects were placed on each plant and a cylindrical screen cage was superimposed over it. Observations were made every 2 days for insect mortality and an estimate of the amount of feeding.

The results of this experiment are shown in table 5. They furnish additional evidence that 1,4-diphenyl semicarbazide is as toxic as derris or lead arsenate to the five species of insects used. In fact, the mortality of the Hawaiian beet webworm, the melonworm, and the southern beet webworm was higher at every concentration than at the same concentration of the standard. A 4-100 spray appears to be as effective as an 8-100 spray, but in most cases there was a definite decrease in toxicity when the concentration was reduced to 2-100. Feeding was slight in most tests except those with the southern beet webworm.

Phytotoxicity Tests

To ascertain whether 1,4-diphenyl semicarbazide can be used with safety on tender foliage, heavy sprays were applied at concentrations of 8-100 and 4-100 to young bean, collard, escarole, lettuce, pumpkin, spinach, swiss chard, and tomato plants growing in direct sunlight in a garden. These tests were made in the spring of the year when the weather was warm but not hot. Six plants of each species were used at both concentrations. All plants were covered during rainy weather and at night to prevent the chemical from being washed off the leaves.

No injurious effects were noticed on any of the plants after one application of the spray, but after a second application, made 1 week later, some chlorosis and browning along the edges of a few leaves was observed on the tomato plants. This injury occurred on foliage sprayed at the 4-100 as well as the 8-100 concentration. No injury to the other species was noted within a 7-day period following the second application of spray.

Table 3.—Toxicity of 1,4-diphenyl semicarbazide as compared with a standard insecticide when applied as sprays to potted plants infested with nearly full grown larvae of several insects

Insect	Concentration of insecticide	Tests	Number	1,4-Diphenyl semicarbazide				Standard insecticide			
				Feeding on sixth day	Kill after 2 days	Percent	Percent	Feeding on sixth day	Kill after 2 days	Percent	Percent
Hawaiian beet webworm	1	3	Slight	17	63	69	Moderate	3	30	42	
	2	do.	do.	37	75	85	Slight	6	41	66	
	4	do.	do.	49	84	97	do.	21	42	64	
	8	do.	do.	61	89	91	do.	36	62	68	
Melonworm	1	2	Moderate	5	64	87	Slight	8	38	70	
	2	Slight	15	95	100	100	do.	6	66	95	
	4	do.	22	98	100	100	do.	18	66	96	
	8	do.	36	100	100	100	do.	24	82	98	
							Lead arsenate				
Southern armyworm	1	3	Slight	1	15	54	Moderate	3	31	65	
	2	do.	do.	19	53	85	Slight	18	66	91	
	4	do.	do.	56	93	99	do.	65	97	100	
	8	do.	79	99	100	100	do.	84	99	100	
Southern beet webworm	1	3	Moderate	7	14	47	Slight	1	10	21	
	2	do.	do.	4	27	46	do.	4	15	29	
	4	do.	24	59	88	88	do.	15	43	69	
	8	Slight	7	54	86	86	do.	18	53	69	
Greenhouse leaf tier	8	1	Slight	73	100	100	Slight	67	100	100	

Field-Laboratory Tests

In the potted-plant tests it had been observed that on leaves sprayed with an effective concentration of 1,4-diphenyl semicarbazide most of the feeding took place the first night, although some of the insects did not die until the sixth day. It was therefore assumed that these tests showed the toxicity of the compound only during the first 24 hours. Another type of test was desirable to check the possibility of a change in the toxicity of the residue after it had been exposed for several days to such weather factors as sunlight and air currents. This information was obtained by cutting leaf samples from sprayed plants in the garden at 2-day intervals and feeding them to fifth instars of the melonworm and southern armyworm in Petri dishes. Parallel tests were made with lead arsenate and derris. These tests were also made in the spring of the year. No information was obtained on the effect of rainfall, as the plants were protected during showers and at night.

The spray mixtures were prepared by grinding the samples to a paste in a mortar with an equal weight of bentonite and a small amount of water and then diluting to a concentration of 8 pounds per 100 gallons. Sodium lauryl sulfate was also used in the sprays applied to collards to make them spread on the waxy surface of the leaves, but no spreading agent was required on the pumpkin leaves. Applications were made with a knapsack sprayer to seven plants until the large droplets on the leaves began to coalesce. As soon as the spray was dry and on the second, fourth, sixth, eighth, and tenth days thereafter, six leaves were picked at random and placed individually in Petri dishes with five insects. On the second, third, fourth, and fifth days after sampling the dishes were examined for dead insects and feeding.

The results of these tests, which are given in table 4, show clearly that 1,4-diphenyl semicarbazide did not lose its toxicity when exposed to sunlight and air currents for 10 days. As in the previous tests, the melonworm was still the more susceptible insect, a better kill being obtained in 3 days than with the southern armyworm in 4 days. The compound was superior to the derris standard in every test with the melonworm, and in some cases it was more effective against the southern armyworm than was lead arsenate. Feeding by the southern armyworm was confined to several small holes in each leaf section, but somewhat heavier feeding was recorded in the melonworm tests with both compounds. In no case was feeding heavy enough to cause severe damage.

Field Tests against Bean Leaf Rollers

In Florida the bean leaf roller is a serious pest of beans during the fall of the year. A practical experiment was therefore made on a row of bean plants heavily infested with first, second, and third instars of this insect. A spray using 6 pounds of 1,4-diphenyl semicarbazide and 6 pounds of bentonite to 100 gallons of water was applied with a 3-gallon knapsack sprayer until the droplets on the leaves began to coalesce. When the row was examined 4 days later, no living bean leaf roller larvae were found.

Table 4.—Results of tests with leaf samples taken at 2-day intervals from plants sprayed in the garden with 1,4-diphenyl semicarbazide and with the standard insecticide and fed to nearly full grown larvae of the southern armyworm and the melonworm in Petri dishes

Insect	Time between spraying and sampling days	1,4-Diphenyl semicarbazide				Standard insecticide				Check (unsprayed)			
		Feeding after 2 days	Kill after 3 days	Feeding after 4 days	Kill after 4 days	Feeding after 2 days	Kill after 3 days	Feeding after 4 days	Kill after 4 days	Feeding after 2 days	Kill after 3 days	Feeding after 4 days	Kill after 4 days
Melonworm	0	Moderate	30	86	93	Moderate	20	56	90	Heavy	0	3	20
	2	do.	3	53	80	do.	6	13	23	do.	0	0	3
	4	do.	40	86	93	do.	3	16	43	do.	3	10	23
	6	do.	26	76	100	do.	16	20	26	do.	6	6	23
	8	do.	30	100	100	do.	0	30	60	do.	3	13	23
	10	do.	20	90	96	do.	3	33	63	do.	0	3	10
						Derris				Percent	Percent	Percent	Percent
Southern armyworm	0	Slight	7	33	60	Slight	0	3	20	Heavy	0	0	0
	2	do.	10	33	57	do.	0	7	27	do.	0	0	0
	4	do.	23	30	63	do.	13	27	60	do.	0	0	0
	6	do.	10	17	33	do.	7	17	37	do.	0	0	0
	8	do.	17	37	57	do.	17	47	80	do.	0	0	0
	10	do.	37	50	73	do.	10	23	do.	0	0	0	0
						Lead arsenate				Percent	Percent	Percent	Percent

Tests against Cockroaches, Termites, and the Rice Weevil

1,4-Diphenyl semicarbazide was also tested against the American cockroach, termites, and the rice weevil. Only preliminary tests were made with each species, as the results were not sufficiently promising to encourage further work.

The tests on cockroaches were made by dusting a 6-inch sheet of filter paper and placing 10 to 15 adult insects on it in the bottom of a 7-inch battery jar. The inside of the jar was greased with vaseline to prevent the roaches from escaping. No mortality occurred in 3 days when a deposit of 310 micrograms per square centimeter was used. In the same period of time a sodium fluoride deposit killed 70 percent of the roaches.

A technique devised by Hockenyos (1) was used in the tests against termites. Tests were made at concentrations of 1-1,000, 1-3,000, and 1-5,000, but the mortality was never greater than 19 percent in 3 days. A phenothiazine standard killed all the insects in 2 days at the 1-1,000 concentration.

In the tests against the rice weevil a sample of wheat was coated with the chemical (1 part by weight of chemical to 1,000 parts of wheat) by shaking the two together in an Erlenmeyer flask, and then placed in a Petri dish with 25 weevils. A mortality of 28 percent resulted in 4 days. However, in similar tests with derris and lead arsenate standards at the same concentration only 20 percent of the weevils were killed.

Summary

Several types of toxicity tests were made with 1,4-diphenyl semicarbazide. When dusted on foliage and placed in Petri dishes, the chemical was highly toxic to nearly full grown larvae of the greenhouse leaf tier, the Hawaiian beet webworm, the melonworm, the southern armyworm, and the southern beet webworm and repellent to larvae of the Colorado potato beetle and the diamondback moth, but ineffective against the cabbage looper, the cross-striped cabbage worm, and the imported cabbage worm. It was about as toxic as the standard insecticide to the Hawaiian beet webworm and the southern armyworm, but more toxic than the standard to the melonworm and the southern beet webworm.

In spray tests on potted plants the compound was as toxic as the two standard insecticides to the five species that were found highly susceptible in the Petri-dish tests. A 4-100 spray was as effective as an 8-100, but in general there was a decrease in toxicity when the concentration was reduced to 2-100. The deposit showed no loss in toxicity after exposure to weathering for 10 days. Spray applications, at concentrations of 8-100 and 4-100, to young bean, collard, escarole, lettuce, pumpkin, spinach, swiss chard, and tomato plants growing in a garden, caused some chlorosis and browning on the tomato plants after the second application, but no injury that was noticeable on the other species.

In preliminary tests the compound was not sufficiently toxic to the American cockroach, a species of termite, and the rice weevil to encourage additional testing.

Literature Cited

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